U. S. Naval School of Aviation Medicine

U. S. NAVAL AIR STATION
PENSACOLA, FLORIDA

RESEARCH REPORT

JOINT RESEARCH REPORT NO. NM 001 088.01.01

STIMULATION OF ERYTHROPOIESIS IN NORMAL ADULT RATS BY A NON-PROTEIN EXTRACT OF PLASMA OF ANEMIC RABBITS

California Institute of Technology
and
U. S Naval School of Aviation Medicine

Navy—PPO CNATRA, Pensacola Fla

U. S. NAVAL SCHOOL OF AVIATION MEDICINE
U. S. NAVAL AIR STATION
PENSACOLA, FLORIDA

JOINT PROJECT REPORT

California Institute of Technology under Contract Nonr-220(09)
Office of Naval Research, Project Designation No. NR 102 007
U. S. Naval School of Aviation Medicine
and
The Bureau of Medicine and Surgery
No. NM 001 027.01.31

STIMULATION OF ERYTHROPOIESIS IN NORMAL ADULT RATS BY A NON-PROTEIN EXTRACT OF PLASMA OF ANEMIC RABBITS

Report by

Henry Borsook, M. D.
Captain Ashton Graybiel, MC, USN
Geoffrey Keighley, Ph.D.
and
Emanuel Windsor, Ph.D.

Approved by

Professor Henry Borsook
Kerckhoff Laboratories of Biology
California Institute of Technology
and
Captain Ashton Graybiel, MC, USN
Director of Research
U. S. Naval School of Aviation Medicine

Released by

Captain James L. Holland, MC, USN
Commanding Officer
U. S. Naval School of Aviation Medicine

20 August 1953

SUMMARY

Experiments are described in which a protein free extract of plasma obtained from anemic rabbits was injected into healthy young rats. The resulting erythropoietic response was comparable to that induced by hypoxia. The findings suggest that a humoral factor was at work which was capable of disturbing the physiological mechanisms which establish the equilibrium between red blood cell formation and destruction. The far reaching significance of these findings are apparent.

INTRODUCTION

The experiments reported here were suggested by the findings in two, at the time, unrelated and independent lines of investigation. One is the increase in hemoglobin and erythrocytes at high altitude (low oxygen tension). The other is the stimulation of amino acid incorporation in vitro into the proteins of rabbit reticulocytes by extracts of liver and plasma described by Borsook and his associates (1). Graybiel and Drabkin (2) proved that in the erythropoietic acclimatization to low oxygen tension heme (and inferentially hemoglobin) synthesis is accelerated. Among the several interpretations of this fact is that erythropoiesis is regulated by a humoral factor: All other things being equal, an increase in the humoral factor causes an increase in erythropoiesis, and vice versa. It was possible that the factor (or factors) in liver or plasma which stimulates amino acid incorporation (protein synthesis) in reticulocytes might be the humoral factor, related to it, or function like it.

The factors found to stimulate amino acid incorporation into rabbit reticulocyte proteins fall into three categories. One consists of certain amino acids: histidine, leucine, phenylalanine, tryptophane and valine. A second we have designated as arbohydrate metabolism factors; these are glucose plus either adenosinetriphosphate, diphosphopyridine nucleotide, or triphosphopyridine nucleotide. Deproteinized extracts of liver and plasma are in a third class since their effects cannot be ascribed to either amino acids, glucose, or any of the known cofactors, vitamins, or common metabolites they contain. It seemed unlikely that in normal animals feeding or injection of glucose and cofactors could have any significant effect. Accordingly, we tested the effects of increasing the amounts of the stimulating amino acids in the diet and of injecting extracts of liver and of plasma.

The following amino acid supplement to the normal ration was prepared. To 265 gm. of dry chow powder was added 0.7 gm. of L-tryptophane and 2.8 gm. each of L-histidine, L-leucine, L-lysine, L-phenylalanine, and L-valine. Fifty mg. of supplemental amino acids per 100 gm. of body weight in the form of

the mixture was the only food given the animals at night so that they ate all of it. In the day time the usual laboratory feed was available to them ad lib. The group consisted of 5 male and 5 female normal adult rats. At the end of 6 weeks there was no significant change or trend in the amount of hemoglobin or the erythrocyte count of any of the animals.

A preliminary experiment with a deproteinized liver extract was carried on for 11 days; positive (or inhibitory) effects did not appear. Positive results were obtained, however, with deproteinized plasma and these experiments will now be described.

FIRST SERIES

Procedure -- The plasma extract was prepared as follows. One ml. of a 2.5 percent solution of phenylhydrazine hydrochloride (neutralized) was injected subcutaneously into adult rabbits. After seven daily injections the animals were anemic and 85-95 percent of the erythrocytes were reticulocytes. Blood was collected by bleeding from the ear vein or by cardiac puncture; coagulation prevented by heparin, 20 mg. of sodium heparin per 100 ml. of blood. The plasma was pipetted off after centrifugation, its pH brought to 5.5 with HCl, and then it was heated in a water bath for 15 minutes. A volume of redistilled water equal to that of the plasma was added to the coagulum, stirred thoroughly, and the suspension was boiled for 5 minutes, then filtered. There were three such washings of the coagulum. The washings and original filtrate were concentrated in vacuo (with heat) to the original volume of the plasma. This solution still contains a small amount of protein (denatured) and is usually strongly opalescent.

Rather than test the extract on anemic animals by comparing rates of regeneration of hemoglobin and red cells against controls, it was decided to use normal animals. This constituted a far more rigid test inasmuch as an increase in erythropoiesis would indicate that the usual controls had been altered. Sprague-Dawley rats were used. Experimental and control groups were litter mates matched for age, sex, weight, hemoglobin and red cell concentration. The animals were 3-6 months old at the beginning of the experiment and their weight ranged from 250-450 gm. Two ml. of the extract per 100 gm. of body weight was injected subcutaneously daily. The animals ate normally, did not lose weight, and appeared healthy after 4 weeks of such injections.

In these experiments, strictly comparable control groups (see below) received daily injections of 2 ml. per 100 gm. of body weight of Krebs' solution (isotonic saline containing 100 mg. percent glucose).

Blood was collected twice weekly for measurement of hemoglobin and hematocrit volume. To draw the blood the rat was wrapped in a towel with its tail protruding; the tail was notched with a razor blade so that it bled freely. Blood was drawn up directly into the measuring pipettes. The wound closed spontaneously.

The hemoglobin determination was a modification of that of Drabkin and Austin (3). To 0.020 ml. of blood was added 4 ml. of water. One drop of 2 percent $K_3Fe(CN)_6$ was added to the hemolyzed blood and allowed to stand for 10 minutes. Then 0.5 ml. of 0.1 percent KCN was added and diluted to 10 ml. with water. The photometric reading was made at 540 mμ against a reagent blank. The standard was a pure cyanmethemoglobin solution treated as above. The hemoglobin concentration in the standard was computed on the basis that its nitrogen content is 16.7 percent. The results are expressed as gm./100 ml. blood.

The hematocrit was measured by a modification of the method of Van Allen (4). Blood was drawn up into a Van Allen pipette to the top of the scale, the outside of the tube wiped clean, 1.8 percent potassium oxalate was drawn up half filling the bulb, and the ends closed tight with a rubber band. The pipette was centrifuged at 825 g for 15 minutes, then turned through 180° and centrifuged another 15 minutes. The volume of packed cells was read directly as percent.

Plasma volume was determined by a modification of the method of Wang and Hegsted (5). The animal was brought under light ether anesthesia, the front of the neck shaved and washed with alcohol. A 2 cm. incision was made from the clavicle to the mandible, the jugular vein exposed by blunt dissection, into which was injected 0.4 ml. of 0.1 percent Evan's Blue in 0.9 percent sodium chloride. Five minutes later 0.5 ml. of blood was withdrawn by cardiac puncture into a syringe moistened with a heparin solution. To 0.4 ml. of blood so drawn was added 3.6 ml. of 0.9 percent sodium chloride mixed gently and the suspension then centrifuged 15 minutes. The color of the supernatant solution was measured against plasma diluted 10 times as a blank. The photometric reading was made at 620 mμ and compared with a standard of Evan's Blue (10 μgm per ml. in 0.9 per sodium chloride). The results were expressed as ml. per 100 gm. of body weight.

Results -- The changes observed in hemoglobin and hematocrit values are given in tables 1-4. No significant changes were noted until one week after beginning the daily injections of plasma filtrate. Then, both the hemoglobin and hematocrit values increased proportionally in the experimental animals for several weeks, the males responding more slowly than the females. This is shown by the absolute values of the average increases ($\triangle$)

in the experimental animals as compared with the controls and in the statistical quantities t and P*. In the control animals there were no statistically significant changes throughout the experimental period.

The possibility was tested that the increases in hemoglobin and hematocrit volume in the animals receiving the plasma filtrate might be due to hemoconcentration. Plasma volume was determined on experimental and control groups of males and females approximately 4 weeks after the beginning of the injections. The results summarized in table 5 show that the blood volume was not significantly different in the experimental than in the control groups and that, if anything, the experimental animals had a somewhat larger blood volume. Since the hemoglobin and hematocrit values are concentrations, larger blood volume tended to reduce these values. It is clear that the increases observed in the experimental groups could not have been an expression of hemoconcentration.

SECOND SERIES

An independent assay of the plasma filtrate was carried out at the U. S. Naval School of Aviation Medicine in Pensacola, Florida.**

Procedure -- One assay was carried out on male and the other on female rats of the Sprague-Dawley strain. There were 4 pairs of animals in each series. Each pair were litter mates, one serving as the experimental animal the other as control. In the males, the experimental subgroup received daily injections of plasma filtrate for 7 weeks and the control subgroup received Krebs' solution. The dose in each instance was 5.0 ml. subcutaneously. Measurements on the blood were made at intervals before, during, and after the period of injections. The procedure in the case of the females was the same except that they were sacrificed after receiving injections for one month for the purpose of studying the bone marrow.

Two days were usually required to carry out the blood measurements on each group, but for statistical purposes the figures were lumped. The determinations were carried out on about 0.3 ml. of heparinized blood. This was obtained by amputating a wafer-thin section of the rat's tail with an especially

*P denotes the probability of the difference from the zero time value occurring by random sampling. A value of P of less than 5 percent is usually considered as statistically significant, and less than 1 percent as highly significant.

**We are indebted to Mr. James Colehour, head of the clinical laboratory, for his cooperation and to Miss Mary McPhaul and James Herbert Wagner, SN, USNR for technical assistance.

designed guillotine. The methods described above were used in measuring the hemoglobin and hematocrit. The reticulocytes were stained and counterstained respectively with Azure II and Wright's stain.

Results -- The chief findings are summarized in table 6 and graphically illustrated in figure 1. The results are clearly cut, both in the male and female animals. With the exception of the initial reticulocyte counts, the values for the control animals in the male group were quite uniform throughout the 14 week period. The failure of these values to increase in young growing rats may have been due to blood loss. In contrast to the controls, the values in the case of the experimental animals showed a rise shortly after the administration of the plasma extract and a fall to control level shortly after the injections were discontinued. Although the magnitude of the increase was not great it was significant, partly because of the temporal relationships which suggest cause and effect and partly because the increase was over and above normal values.

In the case of the females, the magnitude of the response was greater than in the case of the males. The spread between control and experimental values was exaggerated because the blood loss produced a noticeable effect in the control animals.

At the time the female rats were sacrificed, the marrow from the femurs was removed and smears prepared which were stained with May-Grinwald stain. The granulocytic series was counted as one class and the erythroid elements in three classes. The results, summarized in table 7, reveal insignificant differences between the control and experimental subgroups.*

DISCUSSION

The important question is whether our results can be accepted as proof of the presence of an erythropoietic factor in the plasma filtrate. The evidence although indirect otherwise fulfills rigid criteria of erythropoietic activity. The increase in concentration of erythrocytes and hemoglobin in the peripheral blood can only be explained on the basis of hemoconcentration, mobilization of erythrocytes, or increased production. Hemoconcentration was ruled out in the series of experiments where plasma volumes were measured; indeed, the findings suggested that, if anything, the experimental animals had a larger blood volume than the control animals. Mobilization of erythrocytes from spleen or other organs is an unlikely explanation because of (1) the magnitude and long persistence of the increase and (2) the absence of hemoconcentration.

*We are indebted to LTJG James H. Berrian, MSC, USN for this study on the bone marrow.

That increased production of erythrocytes and hemoglobin occurred is suggested not only by the increase in concentration of these elements in the peripheral blood, but also by the increase in reticulocytes and by the overall pattern of the changes in the blood. Reticulocytosis was regularly observed. In Series II the increase in reticulocytes was nearly as great in the control as in the experimental animals, probably because of blood loss. This may account for the failure to observe any difference in erythropoietic activity in the marrow between the control and experimental female rats. The most convincing evidence that the plasma filtrate caused reticulocytosis is seen in the notable decrease in number when injections were discontinued in the male experimental subgroup (figure 1).

We were impressed with the remarkable similarity between the blood changes observed in these animals and in animals subjected to reduced oxygen tensions. As far as we are aware (6), this is the first proof that a tissue extract can produce erythropoiesis in normal animals comparable to that induced by hypoxia. The experiments of Erslev (7) and Reismann (8) are of interest in this connection. The former observed an erythropoietic response in normal rabbits when injected with large amounts of plasma from anemic animals. Resimann's observed erythropoiesis in a parabiotic rat breathing normal air while the partner breathed a low oxygen gas mixture. Both of these experiments as well as our own strongly suggest that a humoral factor is at work.

It is interesting that the stimulating factor (or factors) is not destroyed by fairly prolonged boiling (15 min.) at pH 5.5 under which conditions nearly all the protein in plasma is coagulated. Some protein--material precipitable by 7 percent trichloroacetic acid--remains in solution. As we have no information at present on the chemical nature of the stimulator, it would be premature to exclude the uncoagulated protein as the factor. The factor stimulating amino acid incorporation remains in solution in 7 percent trichloroacetic acid. If the erythropoietic factor behaves similarly it is certainly not a protein, as conventionally defined. Whether or not these two factors are the same or related is under investigation.

It is not to be inferred that a similar result could not have been obtained with plasma extracts of animals with normal blood. The factor stimulating amino acid incorporation into rabbit reticulocyte proteins is present in the blood of all normal animals tested for this factor, in their erythrocytes, and in rabbit reticulocytes. Investigations are now planned to determine whether the erythropoietic factor is present in the plasma and red cells of normal blood of different animals. The isolation of the eyrthropoietic factor is in progress.

BIBLIOGRAPHY

1. Borsook, H., Deasy, C. L., Haagen-Smit, A. J., Keighley, G., and Lowy, P. H. Incorporation in Vitro of Labeled Amino Acids into Proteins of Rabbit Reticulocytes. J. Biol. Chem., 196:669, 1952.

2. Drabkin, D. L. and Graybiel, A. Production of Red Cells in Acclimatization to Altitude--Comparative Study of in Vitro Systems in Biosynthesis of Hemoglobin. Fed. Proc., 10:177, 1951.

3. Drabkin, D. L. and Austin, J. H. Spectrophotometric Studies; Spectrophotometric Constants for Common Hemoglobin Derivations in Human, Dog, and Rabbit Blood. J. Biol. Chem., 98:719, 1932.

4. Van Allen, C. M. J. Clin. Med., 10:1027, 1925.

5. Wang, C. F. and Hegsted, D. M. Determination of Blood and Plasma Volumes, Thiocyanate Space, and Bromsulfalein Clearance in Rats. Amer. J. Physiol., 156:227, 1948.

6. Grant, W. C. and Root, W. S. Fundamental Stimulus for Erythropoiesis. Physiol. Rev., 32:449, 1952.

7. Erslev, A. Humoral Regulation of Red Cell Production. Blood, 8:349, 1953.

8. Reissmann, K. R. Studies on the Mechanism of Erythropoietic Stimulation in Parabiotic Rats during Hypoxia. Blood, 5:372, 1950.

This work was also supported in part by a research grant from the National Institutes of Health, United States Public Health Service, and by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

TABLE 1

FEMALES EXPERIMENTAL

Rat	Zero time		1 week		2 weeks		3 weeks	
	Hb	Ht	Hb	Ht	Hb	Ht	Hb	Ht
1	15.2	49	15.4	50.5	16.8	52.5		
2	15.9	50	16.9	54	17.8	57.5	19.1	64
3	14.4	47.5	15.6	51	17.7	56.5	18.0	58
4	15.3	48	16.6	55	18.1	60.0	29.4	66
5	14.7	48.5	13.9	44	14.1	48.5	15.6	50
6	15.0	46	16.2	52.5	18.1	60		
7	15.9	50	13.0	42	18.2	59.5		
8	14.8	47	15.1	52	17.6	57		
9	15.1	47	16.9	54	17.7	58		
10	15.1	47	18.0	54.5				
11	14.6	45	15.4	50	17.3	56		
12	13.0	41	14.5	47	16.4	54.5		
13	14.2	45	15.6	52.5	17.0	54		
14	14.8	43.5	16.2	55.5	17.5	57		
15	13.8	42	16.2	55	17.8	59		
Avg.	14.8	46.5	15.7	51.3	17.3	56.4	18.3	59.5
S.D.	±0.56	±2.7	±1.26	±4.0	±1.01	±3.15	±2.05	±7.2
Δ			+0.9	4.8	+2.5	9.9	+3.2	11.0
t			2.38	3.8	7.41	9.16	2.99	3.02
P			<5%>1%	<1%	<1%	<1%	<5%,>1%	<5%>1%

TABLE 2

FEMALES CONTROLS

Rat	Zero time		1 week		2 weeks		3 weeks	
	Hb	Ht	Hb	Ht	Hb	Ht	Hb	Ht
1	14.3	45	14.2	42	14.5	45.5		
2	13.4	42.5	14.7	43	14.1	45.5		
3	14.8	47.5	14.0	47	15.4	47		
4	14.3	47.5	14.6	47	14.9	47		
5	15.4	46.5	15.4	47	14.1	47	14.4	47
6	14.0	44	14.0	45.5	14.5	44	13.8	45
7	14.0	42	14.8	45.5	14.7	44	14.5	46.5
8	13.8	41	13.6	43	14.1	42	13.4	41
9	15.0	47	14.7	47	15.0	47		
10	15.5	48	15.0	47.5	15.7	46.5		
11	15.2	49.5	15.9	50.5	16.3	51.5		
12	13.9	42	13.2	41	14.6	47		
13	14.8	44	14.8	47	14.6	45		
14	14.7	47	14.8	47.5	13.1	42.5		
15	14.5	44.5	14.1	43.5	14.7	46		
Avg.	14.5	45.2	14.5	45.6	14.7	45.8	14.0	44.8
S.D.	±0.62	±2.6	±0.69	±2.6	±0.75	±2.3	±0.52	±2.7
△			0	0.4	+0.2	0.6	-0.5	-0.4
t			0	0.423	0.793	0.645	0.622	0.824
P				>5%	>5%	>5%	>5%	>5%

TABLE 3

MALES EXPERIMENTAL

Rat	Zero time		1 week		2 weeks		3 weeks		4 weeks	
	Hb	Ht	Hb	Ht	Hb	Ht	Hb	Ht	Hb	Ht
1	14.8	47	14.7	49	16.0	49				
2	15.9	53.5	16.8	56.5	18.2	62	17.4	60.5	17.1	57
3	15.5	49	15.9	51.5	16.0	57.5	17.1	52.5	17.0	54.5
4	14.4	45	12.4	44	14.7	51.5	16.7	57.5	16.9	58.5
5	15.0	51.5	15.5	54	17.0	58				
6	13.8	45	15.0	50	16.6	56	17.0	57.5	17.8	62
7	14.6	50.5	16.4	56	16.7	57	17.6	60.5	18.9	62
8	14.0	44	15.7	50	16.5	57	18.0	62	19.0	60.5
9	13.8	43	16.2	51	17.2	59.5	17.9	63	18.5	59.5
10	12.6	41.5	15.2	51	16.2	52.5	17.3	60	18.5	59
11	13.0	43	15.5	51.5	16.3	53	17.2	56	18.9	59
Avg.	14.30	46.63	15.39	51.31	16.49	55.72	17.35	58.83	18.06	59.11
S.D.	±1.0	±3.5	±1.55	±3.7	±0.87	±3.8	±0.42	±3.3	±0.86	±1.9
△			1.09	4.68	2.19	9.09	3.05	12.23	3.76	12.47
t			1.98	3.29	5.47	6.14	8.71	8.43	8.95	9.81
P			>5%	<1%	<1%	<1%	<1%	<1%	<1%	<1%

TABLE 4

MALES CONTROLS

Rat	Zero time		1 week		2 weeks		3 weeks		4 weeks	
	Hb	Ht	Hb	Ht	Hb	Ht	Hb	Ht	Hb	Ht
1	14.2	47	14.4	46	15.9	50				
2	15.1	50	14.7	46.5	16.3	52				
3	15.2	48	15.3	46	15.2	49				
4	16.5	51	16.5	51	13.4	44.5	10.1	40.5	13.1	44
5	16.3	50	15.7	46	15.5	47	15.2	49	14.0	45
6	15.0	46	14.8	46			14.5	46	14.5	45
7	16.2	52	16.0	51.5	15.8	54	15.2	49.5	14.4	43
8	14.6	42	14.3	47	14.6	45	16.1	52.5	15.7	48.5
9	14.2	46	14.0	47.5	14.5	47	15.6	47.5	17.1	47.5
10	14.8	46.5	13.5	45.5	14.3	47	14.9	48.5	14.0	44.5
11	14.3	46	14.4	51	15.6	49	15.9	52	15.2	48
12	12.9	44	15.1	46.5	16.0	49	12.2	43	14.8	50
13	14.7	45	15.4	48.5	15.3	48	17.5	54	16.1	48
Avg.	14.92	47.1	14.93	47.6	15.20	48.4	14.72	48.25	14.89	46.85
S.D.	±0.97	±2.9	±0.84	±2.2	±0.83	±4.4	±2.2	±4.2	±1.17	±2.1
△			0.01	0.6	0.30	1.3	-0.2	1.15	≈0	-0.25
t			≈0	0.6	0.82	0.88	0.26	0.78	≈0	0.23
P				>5%	>5%	>5%	>5%	>5%		>5%

TABLE 5

BLOOD VOLUME OF EXPERIMENTAL AND CONTROL ANIMALS TWENTY-SEVEN DAYS AFTER BEGINNING OF INJECTIONS

	Weight	Hb. gm./100 ml. blood	Ht. percent	Blood volume ml./100 gm. body weight	Hb. x B.V.	Ht. x B.V.
			EXPERIMENTAL FEMALES			
	340	19.3	62.5	7.4	143	536
	296	18.6	61.5	7.5	139	461
	302	19.9	66.5	8.6	171	572
	284	16.9	53.5	6.6	111	353
Avg.		18.7	61.0	7.52	141	459
			CONTROL FEMALES			
	292	14.2	42.5	5.9	84	251
	284	14.7	44	6.2	91	273
	308	14.7	47	7.2	106	338
	358	13.8	42.5	5.9	81	251
Avg.		14.35	44.1	6.30	90	278
			EXPERIMENTAL MALES			
	330	17.1	57	8.4	144	479
	464	17.0	54.5	5.3	90	289
	368	16.9	58.5	7.5	127	439
Avg.		17.0	56.6	7.06	120	399
			CONTROL MALES			
	406	13.1	44	6.2	81	273
	500	14.0	45	5.9	83	265
	492	14.5	45	5.8	84	261
	476	14.4	48	5.9	85	283
Avg.		14.0	45.5	5.95	83	271

TABLE 6

MEAN VALUES OF BLOOD MEASUREMENTS ON FEMALE AND MALE RATS. THE N IN EACH SUBGROUP IS 4. IN THE EXPERIMENTAL SUBGROUPS THE FEMALES RECEIVED DAILY INJECTIONS OF FACTOR X DURING +1 THROUGH +4 WEEKS; THE MALES DURING +1 THROUGH +7 WEEKS

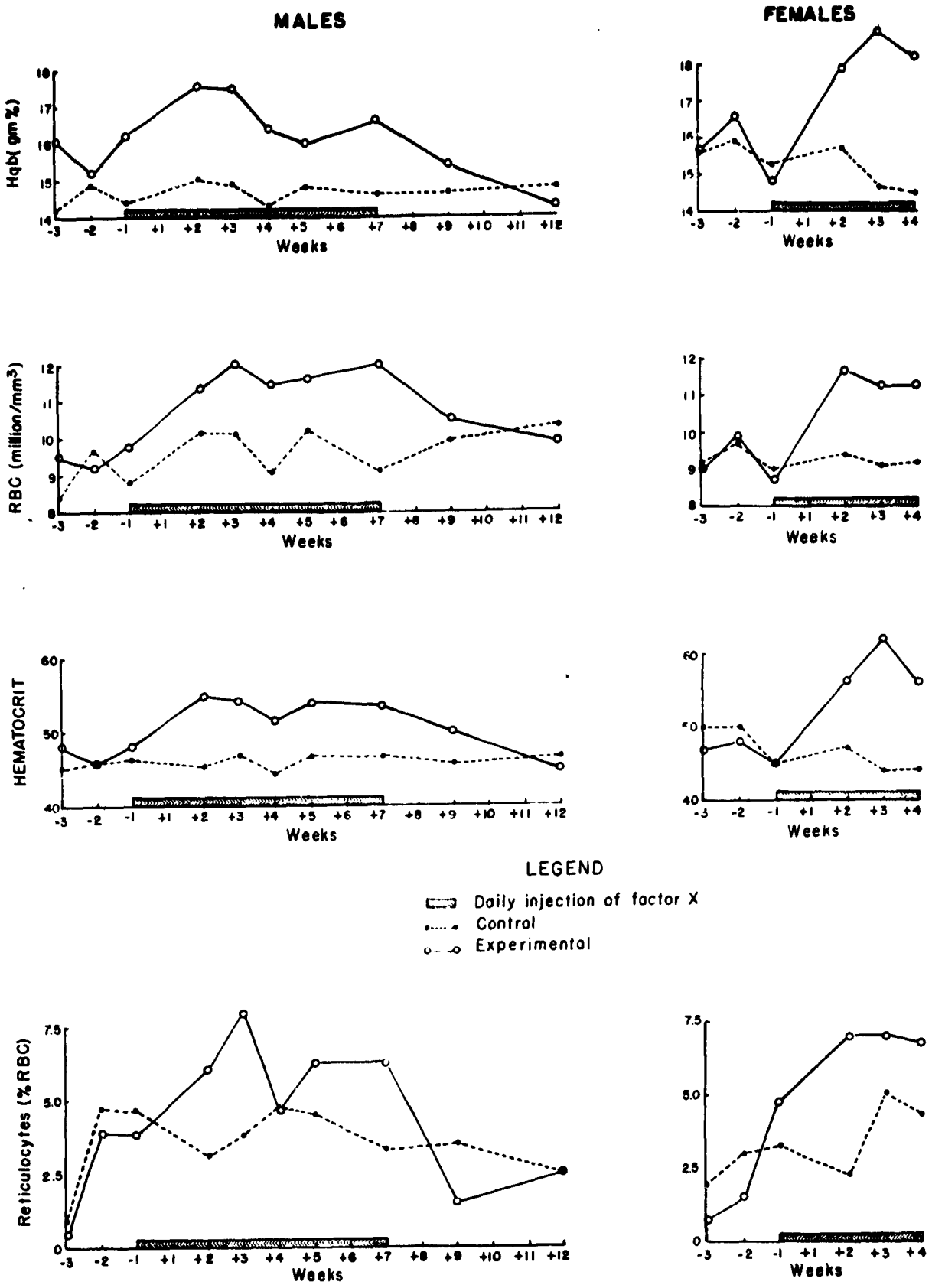
			Weeks									
			-3	-2	-1	+2	+3	+4	+5	+7	+9	+12
Hgb. (gm.%)	Female	Control	15.6	15.9	15.3	15.5	14.7	14.5				
		Exp.	15.7	16.6	14.8	17.9	18.9	18.2				
	Male	Control	14.2	14.9	14.4*	15.0	14.9	14.3	14.8	14.6	14.7*	14.8
		Exp.	16.1	15.2	16.2	17.5	17.5	16.4	16.0	16.6	15.4*	14.3
RBC million per mm^3	Female	Control	9.18	9.70	8.97	9.43	9.07	9.23				
		Exp.	9.07	9.91	8.67	11.70	11.28	11.31				
	Male	Control	8.43	9.68	8.79	10.08	10.14	9.07	10.15	9.01•	9.88	10.28
		Exp.	9.52	9.18	9.76	11.31	12.03	11.47	11.58	11.96*	10.52	9.86
Hema-tocrit	Female	Control	50.0	49.5	45.1	47.2	44.4	44.3*				
		Exp.	47.0	47.7	44.6	56.3*	62.0*	55.6*				
	Male	Control	44.9	45.3	46.4*	45.2*	46.9	44.4*	46.7*	46.5	45.7	46.4
		Exp.	48.1	45.5	47.9	55.1	54.3	51.5*	54.2*	53.7	49.9	45.1
Retics per 1000 RBC	Female	Control	20	30	33	23	50	44				
		Exp.	8	16	48	70	71	67				
	Male	Control	9	47	47	31	38	48	41	33	34	25
		Exp.	6	39	38	61	76	47	63	63	17	26
MCHC	Female	Control	31.4	32.1	33.7	32.8	33.2	33.0				
		Exp.	33.2	34.7	33.0	31.4*	30.8*	33.0*				
	Male	Control	31.7	33.0	31.0•	31.3*	31.8	32.6*	32.2*	31.5	32.3*	34.2
		Exp.	33.2	33.3	33.8	31.8	32.2	30.7*	30.6*	30.9	31.0*	31.5
MCV	Female	Control	54.4	51.2	50.6	50.1	49.2	48.2				
		Exp.	52.1	48.1	51.8	47.9*	53.7*	49.3*				
	Male	Control	53.2	47.0	52.1*	47.6*	46.2	48.5*	47.5*	52.6•	46.3	45.3
		Exp.	50.7	49.7	49.1	48.9	45.3	44.6*	44.8*	45.7*	47.5	45.8
MCH	Female	Control	17.0	16.4	17.1	16.4	16.4	15.8				
		Exp.	17.4	16.7	17.1	15.4	16.8	16.2				
	Male	Control	16.9	15.5	16.0*	15.0	14.7	15.8	14.6	16.9•	15.1*	14.4
		Exp.	16.9	16.6	16.6	15.5	14.6	14.3	13.8	14.2*	15.0*	14.5

* — mean of 3
• — mean of 2

TABLE 7

CELLULAR COMPOSITION OF FEMORAL BONE MARROW OF 8 FEMALE RATS.
THE EXPERIMENTAL GROUP RECEIVED DAILY INJECTIONS OF FACTOR X FOR ONE MONTH

CONTROL GROUP						EXPERIMENTAL GROUP					
RAT NUMBER	PROGRANULOCYTES	NORMOBLASTS	EARLY ERYTHROCYTES	LATE ERYTHROCYTES	% BLASTS	RAT NUMBER	PROGRANULOCYTES	NORMOBLASTS	EARLY ERYTHROCYTES	LATE ERYTHROCYTES	% BLASTS
2	782	37	175	174	33.6	1	830	78	247	210	39.2
4	885	59	266	302	41.5	3	774	28	249	356	46.2
6	950	22	334	196	36.8	5	645	35	248	217	43.7
8	431	45	339	268	60.0	7	1071	23	336	212	53.3
					43.0 av.						45.8 av.



Changing pattern in blood of rats before, during and after administration of factor X. Each point represents the mean values based on four animals (for exceptions see Table 6).

FIGURE 1